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THE EFFECT OF THE PRESENCE OF INSOLUBLE SUBSTANCES ON THE TOXIC ACTION OF POISONS.[1]

RODNEY H. TRUE and C. S. OGLEVEE.

IT has been repeatedly observed in connection with the poisonous action of dissolved substances that the harmful effects are much more marked when the roots of the plants under test are immersed in the solutions than when these are grown in sand cultures which are watered with like or even more concentrated solutions (1). Upon this point the practice advocated by NÄGELI (2) for removing traces of metals from distilled water may be cited. When to distilled water, rendered harmful for algae by the presence of traces of copper, crushed graphite, shredded filter paper, paraffin shavings, and other insoluble substances are added, the copper is removed from the water by some process dependent to a degree upon the amount of surface offered by the insoluble substance.

In connection with experiments carried out in 1902 by TRUE and GIES (3) at the Plant Physiological Laboratory at Woods Hole, Mass., on the modification of the action of poisons by the presence of other compounds, some preliminary experiments were made bearing on the effect exerted by the presence of insoluble materials on the behavior of seedlings of *Lupinus albus*. A concentration of copper sulfate was experimentally determined in which growth for twenty-four hours was possible, though at a somewhat diminished rate. When to duplicate cultures insoluble substances were added, in nearly all cases a marked increase in the growth rate was noted.

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[1] After this paper was prepared, the line of argument and the evidence were submitted to Dr. LYMAN J. BRIGGS, physicist of the Bureau of Soils, U. S. Department of Agriculture. He has most generously given us the benefit of his special advantages.

The same volume of solution was used in each case and the same amount of copper was present in each culture. In some cases a copper-containing solution, in itself harmful, gave a marked stimulation when the insoluble material was added. In other cases the increase in the growth rate was less marked or, in two cases, absent. The general trend of the evidence, however, was clearly in favor of NÄGELI'S conclusion that insoluble bodies in some way remove from action limited quantities of the copper in the solutions.

During the summer session of 1903, C. S. OGLEVEE, of the John Milliken University of Lincoln, Illinois, while working in the Plant Physiological Laboratory at Woods Hole, repeated the experiments above described and extended their scope. He used the radicles of seedlings of *Lupinus albus* as test objects.

The insoluble substances used represented several types of substances, having little in common beyond their insolubility. In making a choice of these the suggestions of NÄGELI were again acted upon. In experimenting with these different insoluble or difficultly soluble materials we attempted to use such quantities of each substance as would offer, as nearly as possible, like surfaces to the solutions. It is improbable, however, that even approximate accuracy was reached on account of the different sizes and shapes of the particles. Clean sea sand of rather coarse texture was used most frequently. It was selected from the cleanest parts of the beach near Woods Hole, boiled for about an hour in dilute hydrochloric acid, then thoroughly washed in fresh water and finally in distilled water.

Powdered Bohemian glass was prepared by crushing up some flasks and beakers that had been cracked or broken. This crushed glass was repeatedly washed in distilled water for a long time before being made use of in the experiment. It is a matter of common information that glass of this type is not strictly insoluble in water, and traces of the more soluble constituents were doubtless present in experiments with this substance.

Filter paper was also used. Cut filters of good commercial grade were shredded into distilled water and digested for a protracted period with several changes of water before use.

Powdered anthracite coal was also tried after a thorough treatment with distilled water. It was noted that gas bubbles were repeatedly found over the surface of the submerged particles of coal and that after being removed by thorough stirring these would again form. This evolution of gas continued during the entire time in which the coal was under observation. The nature of the gas was not determined.

Finely divided pure paraffin of high melting point was also made use of. Investigations made on the effect of paraffin on the electrical conductivity of pure water have shown that paraffin does not noticeably increase the conductivity and seems to be practically insoluble in water.[2]

Owing to the lightness of the paraffin, the finely divided particles floated on the surface of the solutions, but owing to the small spaces between the pieces, the solution rose by capillary action among them and they became, in effect, submerged in the solutions.

At the suggestion of Dr. HENRY KRAEMER, of the Philadelphia College of Pharmacy, unruptured potato starch grains were tried. A stock was freshly prepared from potatoes by crushing and washing out the starch grains, care being taken to avoid other structures from the tuber. The starch thus obtained was dazzlingly white and distilled water in which a portion of the material was allowed to stand for some time gave no starch reaction when treated with iodine. By rubbing up a little of the same starch with a little distilled water a strong starch reaction appeared on adding iodine solution. Careful washing removed all traces of other substances.

The toxic substances receiving most attention in this investigation were those which have been shown to possess great poisonous activity. Copper sulfate, silver nitrate, mercuric chlorid, hydrochloric acid, sodium hydroxid, thymol, and resorcinol may be especially mentioned. The chemicals in the case of the heavy metals were Merck's guaranteed reagent grade. The remaining compounds were recognized makes of chemically pure quality. The solutions were made up with such care as the apparatus of the laboratory permitted, and we believe that the concentrations were usually accurate.

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[2] We are informed that investigations by F. K. CAMERON and LYMAN J. BRIGGS of the Bureau of Soils, U. S. Department of Agriculture, show that high grade paraffin of high melting point, of the sort here used, has but a minimal effect on the electrical conductivity of distilled water in which it is placed.

The method of operation consisted essentially in suspending the seedlings on glass rods in the solutions concerned for twenty-four hours each in each experiment, in such a manner as to immerse the radicles. In nearly all cases the plants were under observation at least forty-eight hours. Never less than four seedlings were used in any experiment and frequently more were employed. The average is taken as the result. The seedlings were placed with the roots in the solutions, care being taken to use plants in like stages of development. Growth was measured from an India ink line placed 15 ^{mm} from the tips of the roots at the beginning of the experiment. During the exposure to the action of the solutions the culture dishes stood in diffused light. The influence of variable factors, such as temperature, was kept track of by check cultures in distilled water.

The water was distilled in an apparatus lined with block tin and was not, therefore, of the highest purity. It was consistently used, however, throughout the season and forms a constant factor in all experiments. Beakers of 300^{cc} capacity and of similar shape were used.

In studying the effect of the presence of insoluble substances on the poisonous activity of the compounds mentioned, a quantity of the sand, filter paper, or paraffin was generally used equal to about one-third of the volume of the solution of the poisonous agent. Thus, in the beakers used for these cultures about two-thirds of the depth of the solution was to be seen *above* the level of the sediment in all but the floating paraffin, in which case the order was inverted.

After marking the roots and introducing them into the solutions under study, the plants were set away for twenty-four hours, at the end of which time they were again measured and the amount of growth noted. Usually, to avoid further complications arising in the case of prolonged experiments, the results due to exposure of twenty-four hours were sought.

EXPERIMENTAL RESULTS.

The first step taken with each toxic agent was to determine approximately the maximum concentration permitting the survival of the plants. This was then followed by a study of the results following the introduction of the particular insoluble substance under study.

The first group of toxic agents tested were exclusively electrolytes, which at the very advanced degree of dilution here seen may be regarded as acting chiefly as ions. The possible presence of a residuum of molecules, especially in the case of $HgCl_2$, is of no particular significance for the present discussion. Concentrations are always cited in terms of parts of a gram-molecule of the substance in question per liter of solution.

Mercuric chlorid.

Preliminary experiments were made to determine the toxic activity of the solution in the absence of insoluble substances. A concentration was then selected at which growth, although greatly depressed, is still possible before death results, and parallel cultures were then made in these concentrations both with and without the insoluble substance. The following table summarizes the results, growth made being indicated in millimeters:

TABLE I.

MERCURIC CHLORID.

1 gram-molecule	Growth in 24 hrs.	Growth in 70 hrs.
18,000 liters	2.0^{mm}	6.5^{mm}
18,000 + 75^{gm} sand	6.0	21.0
20,000 liters	10.0	28.5
20,000 + crushed glass	16.9	40.0
Check in water	12.0	16.0

It is clear that the presence of both sand and crushed glass here modifies very markedly the toxic influence of the mercuric chlorid. This amelioration is evident not only during the first twenty-four hours of the experiment, during which the roots were exposed to the corrosive sublimate solution, but also during the following period of nearly two days, during which the roots were placed in tap water. The course of the check experiment shows that the presence of the insoluble bodies exerts a clearly stimulating effect on the roots. The same may be seen in a less marked degree in the more dilute solution lacking the insoluble substance.

Silver nitrate.

The above methods were applied to silver nitrate in a large number of dilutions, with somewhat less marked results.

TABLE II.

SILVER NITRATE.

1 gram-molecule	Growth in 24 hrs.	Growth in 48 hrs.
JULY 22		
150,000 liters	7.0^{mm}	8.0^{mm}
150,000 liters + sand	17.0	34.0
200,000 liters	13.0	22.0
200,000 liters + sand	16.0	31.0
200,000 liters + starch	13.0	22.0
250,000 liters	10.0	16.0 (weak seedlings.)
250,000 liters + sand	15.0	32.0
Water, distilled	16.0	19.0
Water + sand	12.0	14.0
AUGUST 20		
250,000 liters	16.0	
250,000 liters + sand	16.0	
300,000 liters	16.0	
300,000 liters + sand	18.0	
Distilled water	14.0	
Distilled water + sand	12.0	
AUGUST 17		
350,000 liters	14.0	
350,000 liters + sand	12.0	
350,000 liters + glass	14.0	
350,000 liters + filter paper	19.0	
Distilled water	12.0	
Distilled water + filter paper	13.0	
AUGUST 14		
350,000 liters	10.0	18.0
350,000 liters + sand	17.0	34.0
Distilled water	9.0	15.0

A study of the numerical data shows that in four cases out of ten, in which the active solutions with and without the solids are compared, no increased growth rate appears in the presence of the solid. In three out of four cases marked by the absence of an acceleration, the resulting growth rate agrees with that of the roots in the solutions lacking solids. In one case out of ten there is a retardation seen to follow the introduction of the solid. If we examine the six cases in which an acceleration followed the introduction of the solid, the sum of the differences in favor of the cultures containing the solids is 32^{mm}. Thus, seen collectively, the increase in growth rate due to the presence of the solids is very marked. Since no experiments were made for contrasting the efficiency of the various solids used, we cannot say in how far the differences in the nature or in the quantity of the solids present may be responsible for the differences noted. In some degree, also, slight differences in handling during the experiment and also individual differences in the roots produce slight, uncontrolled effects.

Copper sulfate.

A greater number of experiments were made with copper sulfate than with any other substance. This has an added interest because of NÄGELI'S (**2**, p. 23) experiments with Spirogyra in extremely dilute solution. NÄGELI found that distilled water prepared by means of a copper still may contain enough metal to produce fatal results with Spirogyra. The remedy proposed as a laboratory expedient for making water thus prepared fit for use was to put finely divided insoluble bodies into the water. The results obtained with copper sulfate follow. Although there is considerable repetition and some experiments are not completely carried out, it seems desirable to present all the evidence, showing as it does the range of variation in the results:

TABLE III.

COPPER SULFATE.

1 gram-molecule	Growth in 24 hrs.	Growth in 70 hrs.
JULY 8		
32,768 liters	2.0mm	Dead
32,768 liters + glass	2.0	"
49,152 liters	3.0	"
49,152 liters + sand	4.0	"
Distilled water	12.0	17.0mm
JULY 10		
50,000 liters	1.0	Dead
50,000 liters + sand	14.0	28.0mm
65,000 liters	12.0	28.0
65,000 liters + sand	16.0	32.0
JULY 13		Growth in 48 hrs.
35,000 liters	1.0	Dead
35,000 liters + sand	7.0	12.0mm
40,000 liters	2.0	Dead
40,000 liters + sand	5.0	9.0mm
50,000 liters	2.0	Dead
50,000 liters + sand	11.0	17.0mm
Distilled water	10.0	13.0
Distilled water + sand	8.0	15.0

TABLE III—*Continued*

1 gram-molecule	Growth in 24 hrs.	Growth in 70 hrs.
JULY 16		
35,000 liters	4.0	Dead
35,000 liters + glass	10.0	17.0$^{\text{mm}}$
35,000 liters + filter paper	18.0	32.0
Distilled water	12.0	15.0
Distilled water + glass	9.0	16.0
Distilled water + filter paper	12.0	21.0
Distilled water + glass wool	16.0	29.0
JULY 20		
35,000 liters + starch	12.0	
35,00 liters + glass wool	5.0	
Distilled water	14.0	
Distilled water + sand	12.0	
JULY 24		Growth in 72 hrs.
35,000 liters	3.0	
35,000 liters + paraffin	8.0	
Distilled water	12.0	
JULY 29		
35,000 liters	1.0	
35,000 liters + glass	4.0	
JULY 30		
35,000 liters	1.0	
35,000 liters + paraffin	3.0	
JULY 31		
35,000 liters	1.0	Dead
35,000 liters + garden soil	13.0	19.0$^{\text{mm}}$
Distilled water	14.0	19.0
Redistilled water	15.0	16.0
AUGUST 5		
35,000 liters	2.0	
35,000 liters + sand	7.0	
35,000 liters + paraffin	5.0	
Distilled water	12.0	
AUGUST 10		Growth in 48 hrs.
35,000 liters	2.0	Dead
35,000 liters + 40$^{\text{grms}}$ sand	7.0	Living
35,000 liters + 80$^{\text{grms}}$ sand	10.0	Good condition
35,000 liters + 120$^{\text{grms}}$ sand	16.0	Excellent
35,000 liters + 160$^{\text{grms}}$ sand	13.0	Thrifty
35,000 liters + 200$^{\text{grms}}$ sand	12.0	Thrifty

Several facts seem to be clearly brought out in the above exhibit. Lupine roots are unable to survive an exposure of twenty-four hours to a concentration of copper sulfate in which a molecular weight is dissolved in much less than 60,000 liters—a point which has been repeatedly observed before (3, 4). When, however, to a solution containing nearly or quite twice this amount of the copper salt, finely divided insoluble bodies, like quartz sand, crushed glass, and filter paper are added, the toxic action is so far diminished as to permit not only an increased growth before death takes place but even vigorous survival. In case the amount of copper is diminished, an advantage is regularly found in favor of the cultures containing the solid body.

In the case of the culture containing the garden earth, the effect of a common type of soil containing considerable soluble matter was desired. Of course chemical reactions have entered here, but at this great dilution the copper probably still existed in the ion condition. Here, too, the introduced soluble material may have had its own direct action on the organisms.

The action of paraffin is marked in every case but in a somewhat less degree than in the other cases noted. The paraffin offered some slight difficulty of method which may account somewhat for the fact observed. Although hard paraffin was used, it was found difficult to shave or chop it up into particles of the desired degree of minuteness. Thus less surface was exposed to the solution than in the other substances used. Its lightness, too, tended to buoy it up upon the surface of the solution, and prevent a thorough mingling. This was remedied in part by the tendency of the solution to rise by capillary action among the floating particles. It was also found practicable to hold the paraffin under, to a considerable degree, by means of pressure on the surface of the floating mass.

The effect of solids on growth in the distilled water was tried in control experiments, the general result seeming to indicate that the plants are not favored by this presence. Redistilling from glass, discarding the first half of the distillate and leaving a third of the original volume in the retort, seems to yield a product having no advantage over the water but once distilled.

In all of the toxic substances thus far considered, we have had to do exclusively with electrolytes, therefore, at the high dilutions concerned, with ions. Experiments were made with a few non-electrolytes in order to ascertain whether the ionization of the molecule may be a necessity for the observed decrease in the poisonous action of the presence of insoluble substances.

Non-electrolytes.

Those tested in this connection were phenol, resorcinol, and thymol. The investigations by BADER (**5**) on the conductivity of phenyllic compounds showed that in the substances here concerned no ionization is to be found. We have, therefore, here to do with entire molecules.

TABLE IV.

PHENOL.

1 gram-molecule	Growth in 24 hrs.
AUGUST 5	
200 liters	5.0mm
300 liters	6.5
300 liters + sand	6.0
Distilled water	11.5
AUGUST 7	
100 liters	1.0mm
100 liters + sand	2.0
150 liters	2.0
150 liters + sand	2.0
Distilled water	10.0

An inspection of the results shows no apparent amelioration resulting from the presence of the solid. It should be noted, however, in passing, that compared with the electrolytes used, phenol is a weakly toxic substance, and the plants are apt to make a good growth in solutions containing a comparatively great number of molecules (**9**).

TABLE V.

RESORCINOL.

1 gram-molecule	Growth in 24 hrs.
AUGUST 5	
200 liters	13.0mm
200 liters + sand	10.0
300 liters	10.0
400 liters	10.0
Distilled water	11.5
AUGUST 7	
100 liters	2.0
100 liters + sand	3.0
150 liters	3.0
150 liters + sand	5.0
Distilled water	10.0

In this substance, in concentrations great enough to interfere greatly with the growth rate, the presence of the solid seems to bring about a slightly increased rate of growth, this effect ceasing as the dilution is increased. Here we meet again a compound which is tolerated in considerable concentration by the plant (9).

TABLE VI.

THYMOL.

1 gram-molecule	Growth in 24 hrs.	Growth in 48 hrs.
JULY 30		
2600 liters	0.0^{mm}	0.0^{mm} dead
2800 liters	4.0	9.0^{mm}; 2 dead
2800 liters + glass	14.0	28.0^{mm}
JULY 31		
2600 liters	3.5	2 dead
2600 liters + sand	3.0	3 dead
AUGUST 5		
2600 liters	2.0	
2600 liters + sand	1.0	
2800 liters	4.0	
2800 liters + sand	5.0	
Distilled water	11.5	
AUGUST 7		
2600 liters	0.5	all killed
2600 liters + sand	2.0	all killed
2800 liters	0.5	all killed
2800 liters + sand	7.0	3 living
Distilled water	10.0	

The results with thymol, while not homogeneous, indicate that in a concentration of one mol. weight in 2600 liters of solution no steady advantage accompanies the presence of the solid. When the dilution is somewhat increased, however, such an advantage appears. The time at our disposal was too brief to carry out the study of this compound or others as far as seems desirable. Nevertheless, the preponderance of evidence seems to indicate in thymol, and less clearly in resorcinol, a favoring effect accompanying the presence of the solid (9).

DISCUSSION OF RESULTS.

The general situation developed by the results detailed above may with profit be summarized in order to furnish a clear view of the facts which are to underlie our further consideration.

It appears in general that the presence of a considerable body of certain insoluble substances in solutions of strongly toxic compounds, both organic and inorganic in their nature, be they electrolytes or not, tends to decrease the toxic activity of the solutions in question.

On the whole this ameliorating action is more clearly marked in case the poisonous solutions concerned are dilute solutions of strong poisons than when relatively concentrated solutions of weaker poisons are concerned.

In general, filter paper and potato starch grains exert a more marked modifying action than dense sand, glass, and paraffin.

A number of questions immediately arise concerning the relation in which the active ions or molecules stand to this phenomenon. It seems probable that no electrical phenomena are here necessarily concerned. In the case of electrolytes such a situation might be possible, but in thymol and resorcinol we have to do with electrically neutral bodies.

A more satisfactory explanation seems to lie in another quarter. Chemists have long known that gases form a denser layer over the surfaces of the walls of containing vessels than in the free space away from the walls. Certain solids have the capacity of condensing on their surfaces larger volumes of gases than others, seeming to have specific properties in this direction. For example, platinum in a finely divided form condenses many times its own volume of oxygen. A similar phenomenon has long been known in a practical way in the case of solutions. Many years ago it was noted that bone charcoal has the property of taking certain coloring bodies out of syrups, thereby clarifying the latter products. The investigation of this and similar phenomena has shown that molecules of many dissolved substances are attracted to the surface of charcoal, quartz, and filter paper, when these bodies are placed in these solutions, and in general the greater the state of subdivision to which these insoluble bodies are reduced, the more clearly may this condensation of molecules from the solution be seen. This process by which a layer of greater molecular density is formed on the surfaces of solids immersed in solutions has been denominated by DuBois-Reymond *adsorption* (**6**).

Not all solids have been found to be equally effective as adsorbing bodies and all molecules do not seem to lend themselves with equal readiness to the adsorbing influence of solids (**6**).

Applying this doctrine of adsorption to the results observed in our experiments, we find most points noted capable of explanation. The earlier experiments of NÄGELI (**2**) with Spirogyra in distilled water obtained from copper containers and in other solutions containing extremely minute quantities of heavy metals seem capable of satisfactory explanation. He observed that distilled water containing minute traces of metal could be rendered harmless by placing a quantity of some finely divided insoluble substance in the water. He used paraffin, graphite, filter paper, glass, and other substances, finding that they interfered with the toxic action of the metals present. Doubtless the quantity of harmful ions or molecules adsorbed was so great as to reduce the number of ions or molecules in the free body of the solution below the harmful concentration.

In the case of our lupines this same sort of action is also probably at the bottom of the ameliorating influence seen to attend the mere presence of a finely divided solid in solutions or strongly toxic agents. By drawing a portion of the ions or molecules of the poisonous substance from the free body of the solution and by retaining these molecules or ions in a molecularly denser layer over their surfaces, the solids have removed from the free solutions enough of the toxic substance to decrease its poisonous properties. Since in the case of all of the insoluble substances used, paraffin excepted, the specific gravity is greater than that of water, the solids sink to the bottom of the culture vessel. As adsorption takes place, a somewhat gradual process (**6**), molecules and ions are renewed from the solution above, leaving it actually less concentrated than would be the case were the solutions not provided with the solid. As a result, when the roots of the test plant are introduced into the solution, it is found to have a decreased poisonous action.

In the case of certain of the solids used in these experiments, it seems not at all impossible that a small degree of solution may have taken place. In experiments with crushed Bohemian glass this was doubtless the case, since it has been repeatedly demonstrated that such glass undergoes a slight solution even in cold water. Unfortunately we were not able to make conductivity determinations to check up this factor.

In the case of the starch grains, the potato tubers were crushed in a mortar, care being taken not to grind the material and crush the grains. The starch that settled out on washing was repeatedly washed with distilled water. Distilled water in which the starch grains were allowed to stand gave no trace of the starch reaction with iodine, a reaction which appears in case any considerable number of grains are crushed, exposing the more soluble interior layers. Some hesitation, however, may be felt as to the insolubility of the grains.

The question suggests itself as to whether all insoluble solids exert a like adsorptive action under these conditions (**6**). No attempt was here made to test this question, since it was not practicable under the conditions of experiment given to work with the accuracy necessary in such a study. Furthermore, since adsorption is dependent to a considerable degree on the amount of surface offered by the solid, some way of taking account of this factor would seem necessary. In the case of sand this could be done, but to carry over these values to filter paper and starch grains, consisting of masses of organic substance supposed by some (**7**) to have a submicroscopically porous structure, seemed to be impracticable. The lack, therefore, of any practicable unit, whether of weight or surface, by which to compare the solid substances used, made it best to content ourselves with a rough qualitative method.

In general, the most marked results seemed to follow when filter paper or starch grains were used. Whether this is due to the porous structure and consequently immensely enlarged surface, cannot here be discussed.

Another point of interest in this connection concerns the nature of the contact between the solution and the solid body. In all cases except that of paraffin the solution is capable of wetting the surface of the solid, thus bringing the molecules of solute and solvent into closest relation with the adsorbing surface. In the case of paraffin the situation is different. The solution is not capable of wetting the paraffin even when in as close contact as can be obtained. As a result, a surface of quite another sort is formed, more nearly comparable in some ways to that bounding the surface of the solution where it comes in contact with the air. At the point of contact between the solution and the paraffin, the condition of the solution more nearly resembles the surface film. In this case this film surrounds the particles of submerged paraffin, and any properties found to be possessed by the surface of contact with the paraffin should be in a manner similar to those of surface films. However this may be, the surface of contact with the paraffin seems to be such as to bring about a condensation of molecules with a consequent removal of a portion of the poisonous substance from the free solution and a decrease of the poisonous properties of the solution. This happens, also, in the face of the fact that the paraffin floats and must condense the molecules in the upper part of the culture vessel. It might seem, therefore, that the surface portions of the solution should be markedly more toxic than the lower free portion. If this be so, the ions or molecules seem to be held so firmly by the adsorptive force that they are still in effect removed from the solution so far as the roots are concerned.

AMOUNTS OF TOXIC SUBSTANCE REMOVED FROM THE FREE SOLUTION BY ADSORPTION.

The results seen in the tables above given seem to show that solid bodies are capable of adsorbing molecules or ions of toxic compounds, holding them so closely that in effect they are removed from the solution. We see many cases in which the growth rate is so far accelerated by the presence of the adsorbing solid as to lead one to infer that a very considerable portion of the toxic agent may be adsorbed. In cases it is observed that reduction has so far taken place as to practically relieve the solution of its toxic properties. In some instances the solution, decidedly toxic without the solid, becomes capable of supporting a more than normal growth in the presence of the solid. Here, an abnormal acceleration replaces a marked retardation, judged from the growth rate made in the distilled water check culture. Such instances are seen in mercuric chlorid, $\frac{m}{20,000}$. A retardation is seen in the culture lacking the solid amounting to an average of 2 $^{\mathrm{mm}}$. When sand is added, the acceleration is so great as to bring the average growth rate in twenty-four hours to 16 $^{\mathrm{mm}}$, 4 $^{\mathrm{mm}}$ more than in the control culture. In the case of silver nitrate a number of instances of this sort occur. In case the solution without the solid permits a growth rate above that seen in the control, this increase is sometimes still further augmented by the presence of sand. The results with copper sulfate show a number of instances illustrating these different phases of action.

It is a well established fact of general physiology that many compounds which at certain concentrations act as harmful agents and depress activity become in more dilute solutions accelerators of activity. If dilution be carried on there arrives a point at which all effect ceases, and only the effect of the solvent appears. This has been pointed out by COUPIN (**8**) for certain salts. We may represent the different physiological phases resulting from the progressive dilution of a fatal concentration by a series of terms something like the following. Almost instant death (*a*) is followed by a lengthening period of survival (*b*) with depressed activity when compared with the control in water. This depressant phase is followed by a short phase in which the growth rate approximates the control (*c*). This passes over into the more extended acceleration phase (*d*) marked by activity beyond that seen in the control. This acceleration rises to a maximum and with further dilution falls off, until the solution becomes practically distilled water in its action on the organism (*e*). This may be represented diagrammatically as above (*fig. 1*), growth for the twenty-four hours in this case being indicated on the axis of ordinates, progressive dilution (that is, the degree of dilution) on the axis of abscissas progressing to the right, the growth of controls in distilled water being regarded as unaffected by other external conditions, and the roots used assumed to be in the same phase of

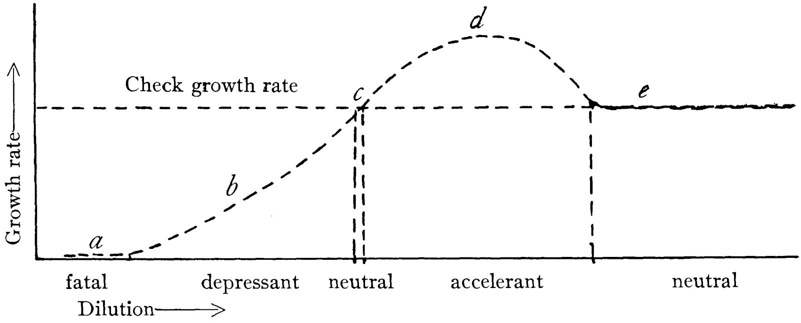

FIG. 1.

development. While it may not be practicable in all cases to work out all features of this generalized scheme with the very toxic salts tested, the most striking points hold.

It is now not difficult to understand a number of phenomena seen in results presented above. By preliminary tests not here detailed a concentration of each substance was found which practically suppressed growth and gave a physiological starting point from which to follow out the action of the poison along the line of progressive dilution and also in the presence of solids. In most cases, the solutions of the toxic compounds lie in the degree of concentration marked by a depressant phase of physiological action. As molecules or ions are removed by adsorption, the effect is virtually one of dilution, and the depressant action becomes less marked; or when adsorption removes a sufficient portion of molecules or ions to bring the solution into the accelerant phase, instead of the depressant, an acceleration above the control rate appears. The degree of acceleration depends on whether adsorption withdraws such a proportion of the molecules or ions as will leave the solution in its maximum accelerant phase or above or below this. It should also be borne in mind that at least theoretically, after the depressant phase has been passed, two quite differing concentrations lying on either side of the maximum may give the same physiological result. Experiments with solutions further diluted will enable one to decide on which side of the maximum the result may lie. Theoretically, a careful study of a long series of slightly differing concentrations should enable us to ascertain relatively how much of a given poison is removed by a given quantity of an adsorbing agent. This can hardly be attempted with justice from the results at hand, since they were obtained under conditions rendering the necessary accuracy of experimentation impossible. Nevertheless, I believe some rough idea may be obtained from the work presented. It will suffice to point out a few instances, since it is manifestly impossible to point out the application of this scheme to all of the results obtained.

In the case of the mercuric chlorid (p. 5) $\frac{m}{18,000}$ is manifestly low in the depressant phase of its action, the introduction of the sand carrying it a manifest step upward, though leaving the situation still in the depressant phase. A solution $\frac{m}{20,000}$ permits a growth rate of 10^{mm}, still less than the control having a rate of 12^{mm}. The depressant phase of dilution is still represented, but is nevertheless but little below the neutral point.

The addition of an adsorbing body therefore should carry it into the accelerant phase. This is the result seen, and the growth of 16^{mm} compared with the check seems to represent a rate probably near the maximum. By making a series of experiments under conditions like those met in this experiment, in which a graded series of solutions of mercuric chlorid alone is used, we should be in possession of a calibrated standard by means of which we could estimate more or less accurately the amount of the toxic molecules adsorbed. Similar methods of interpretation applied to the results with silver nitrate indicate with varying degrees of clearness a similar situation.

The results obtained with copper sulfate illustrate many of the different phases of action. In some cases the presence of the solid served not only to clearly decrease the harmful action of the salt, but even to make survival possible in concentrations otherwise promptly fatal.

In this connection a series of experiments was made to ascertain approximately in how far the quantity of solid substance present might influence the action of a toxic concentration of a salt. For this purpose a series of six cultures was prepared with like quantities of copper sulfate. To five of them prepared sand was added in different quantities. The growth rates seen in Table III, August 10 (p. 7), have been plotted in the following diagram (*fig. 2*). The growth rate in millimeters is indicated as before on the axis of ordinates. The progressive addition of solids is shown on the axis of abscissas. Although, unfortunately, no control culture was made in this case, we may fairly accept 12^{mm} as average growth in distilled water on the date concerned. This value is plotted as the control result. The copper sulfate solution furnishing a basis for this experiment is concentrated enough to kill the roots promptly, usually permitting little growth. The addition of 40^{gm} of sand adsorbs a sufficient amount of copper sulfate to permit half the amount of growth expected of the control culture, and in addition to make possible the survival of the test objects. Nevertheless, a sufficient concentration is still present to leave the solution in the depressant phase of concentration. The doubling of the amount of sand brings the growth rate nearly to the normal rate of the control, a slight depressant action still being seen. The roots, however, survive in good condition, so far as their appearance indicates. The increase of the sand to 120^{gm} results in so advanced a degree of adsorption that the growth rate is thrown clearly into the acceleration stage, perhaps near the maximum. Further additions still further reduce the molecules in the free solution and show a stage of dilution corresponding to the descending stimulus phase. The addition of 200^{gm} of sand shows this reduction of the copper effect at its limit. The free solution is, physiologically, distilled water, so far as the duration of the experiment allows one to judge. Here then the progressive addition of quantities of a solid produces results paralleling the progressive dilution of the toxic medium, the underlying cause of these results being the gradual removal of molecules or ions from the free solution by the insoluble body present.

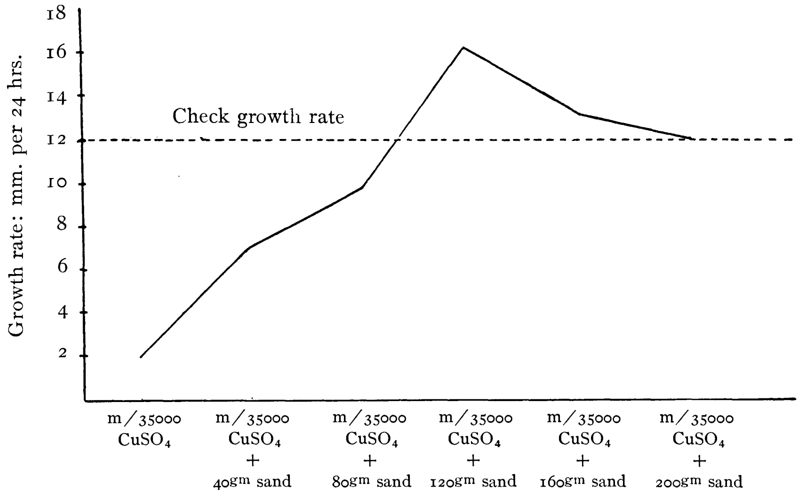

FIG. 2.

In view of the evidence here presented and discussed, it is likely that the process recognized as adsorption plays an important rôle in many problems of plant and probably of animal physiology. Experiments by the water culture method cannot be used as a safe basis for argument concerning soil conditions. The so-called absorptive properties of the soil toward chemical compounds which form a demonstration experiment in the usual elementary course in plant physiology is in considerable part a demonstration of adsorption. Indeed, the whole relation of the plant to its soil environment is very largely influenced by this factor.

It is also probable that in the internal processes of the plant and of the animal, similar forces are in operation, influencing, together with diffusion and osmosis, the movements and distribution of molecules and ions within the organism. For example, it would appear not at all improbable that in cells containing starch grains or other undissolved substances, the distribution of the various molecules and ions of the cell sap is to no insignificant degree influenced by adsorption, and the transfer of molecules or ions from place to place may be modified by this tendency of the walls, starch grains, and other porous but insoluble substances to attach to themselves condensed layers of molecules or ions. Indeed, it would be easy to see in a speculative way many directions in which the processes of life may be affected by the form of activity demonstrated in the above experiments to be in operation under certain common conditions.

In nature the roots themselves and other absorbing organs furnish surfaces for adsorption and the layer of solution about them is molecularly more dense than the free solution. This applies in a much greater degree to minute, submerged forms of life, as bacteria. Absorption into the cell takes place, therefore, in general from a denser layer at the surface of the organism by the continued operation of the energy of adsorption. This surface layer is continually renewed, resulting in an accelerated absorption.

Another relation of importance is seen in the size of the organism. The more minute, the greater would be the efficiency of adsorptive activity. This may aid in accounting for the extreme efficiency of the minuter forms of life in operating on their medium.

Many suggestions occur as to divers other ways in which adsorption may play a part more or less significant in the lives of living things, but to give them value the accumulation of more evidence is necessary. Further studies in this direction are in progress.

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